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Integrated profiling of *Xenorhabdus stockiae* and *Steinernema siamkayai*: Molecular and phenotypic perspectives

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Abstract. We present the characterization of the entomopathogenic nematode isolate LD_CU, and its associated symbiotic entomopathogenic bacterium. BLASTn analysis of the ITS rRNA sequence of the nematode revealed 100% similarity with *Steinernema siamkayai*, confirming conspecificity. The pairwise alignment showed no nucleotide differences with the type population of *S. siamkayai*. Phylogenetic analysis based on ITS rRNA gene sequences supported these findings, placing isolate LD_CU in a monophyletic clade with *S. siamkayai*, which forms a sister clade with *S. huense*, *S. cumgarense*, *S. tami*, and *S. minutum*. Biochemical tests identified the associated bacterial symbiont as *Xenorhabdus stockiae*. The isolate tested negative for oxidase, catalase, nitrate reductase, O-Nitrophenyl-β-D galactopyranoside (ONPG), methyl red, tryptophan deaminase, indole production, ornithine, lysine, citrate, malonate, hydrogen sulfide, acetoin, and phenylalanine deaminase, but positive for urea and esculin hydrolysis, motility, and acid production from glucose fermentation. The bacteria exhibited neutral red adsorption on MacConkey agar and formed brownish pigmented colonies on nutrient agar. Molecular characterization using 16S rRNA gene sequences revealed 100% similarity with *X. stockiae* and 90% similarity with *X. innexi*, indicating significant divergence from other *Xenorhabdus* species. Phylogenetic analysis showed a close relation to *X. stockiae* VP-2016b and distinct differences from *X. innexi* DSM 16336T. Pairwise alignment confirmed no nucleotide differences between the present bacterial strain and *X. stockiae*. This detailed and comprehensive profiling supports the accurate identification of *Steinernema siamkayai* and its bacterial symbiont, *Xenorhabdus stockiae*, and contributes valuable information to the taxonomy and phylogeny of these organisms.

Keywords: Characterization, BLAST, ITS rRNA, 16S rRNA, Entomopathogenic nematode, Symbiotic bacterium, Insect pathology

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Introduction

Entomopathogenic nematodes (EPNs) are natural parasites of insects in their environment. The gut of infective juveniles (IJs) of EPNs contains bacterial symbionts, which are released into the insect hemocoel upon penetration (Machado *et al.*, 2024). This symbiotic relationship, particularly between EPNs of the *Steinernema* genus and their associated *Xenorhabdus* bacteria, is crucial for insect pathogenicity. The *Xenorhabdus* bacteria reside within specialized vesicles in the nematode's gut and are essential for nematode growth beyond the J1 stage (Sajnaga &

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Kazimierczak, 2020). *Steinernema* nematodes inhabit soil environments where they parasitize insects and other small arthropods. Upon entering a host insect, *Steinernema* nematodes release the *Xenorhabdus* bacteria from specialized vesicles near the anterior part of the infective juvenile's gut (Machado *et al*., 2023). These bacteria play multiple roles: they produce antimicrobial substances and secondary metabolites that protect the insect cadaver from scavengers and soil saprobes, while also providing nutrients to support nematode proliferation (Lefoulon *et al*., 2022). The lifecycle continues as the nematodes feed on insect tissue and *Xenorhabdus* bacteria until resources are depleted. At this point, they re-establish their symbiotic relationship and leave the insect cadaver to seek new hosts (Boemare, 2002). Due to their potent insecticidal effect and non-toxicity to vertebrates, steinernematids have been employed as effective biological control agents against soil insect pests (Kenney & Eleftherianos, 2016). Furthermore, studies on these organisms have expanded their utility as biological models in evolutionary biology, symbiotic interactions, and soil ecology (Stock, 2015). The present study focuses on the molecular and phenotypic characterization of *Xenorhabdus* species isolated from *Steinernema* nematode recovered from agricultural soils in Western Uttar Pradesh, India. Molecular characterization of these nematode-bacteria symbionts provides insights into their interactions and symbiotic relationships. This offers potential alternatives to chemical pesticides through the development of biopesticides.

Materials and methods

Nematode isolation

A total of twenty soil samples (n = 20) were collected from agricultural fields in the Muzaffarnagar district of Uttar Pradesh, India (29° 28' 21.6552'' N, 77° 42' 30.6324'' E, 249 m above sea level). Each sample (250 g) was collected from around the rhizosphere of sugarcane fields at a depth of 20 cm, placed in well-labeled polythene bags, and transported to the laboratory for EPN isolation. EPNs were isolated from these soil samples using the *Galleria* soil baiting technique (Bedding & Akhurst, 1975) and the White trap method (White, 1927). The infective juveniles (IJs) of the isolated EPNs were stored in tissue culture flasks at 11°C for further use. One sample tested positive for EPN infection. Additionally, free-living nematodes belonging to the genera *Acrobeloides* and *Distolabrellus* were recovered during soil baiting, and are currently undergoing initial characterization.

Identification of EPNs

The isolated nematodes were molecularly characterized by sequencing their internal transcribed spacer (ITS1-5.8S-ITS2) rRNA gene markers. Briefly, genomic DNA (gDNA) was extracted from IJs using a gDNA extraction kit (Qiagen, Germany) following the manufacturer's instructions. The ITS rRNA gene was amplified with the primers described by Vrain *et al*. (1992). Amplified products (5 µL) were electrophoresed on an agarose gel and subsequently Sanger sequenced by Bioserve Ltd. (Hyderabad, India). The assembled ITS rRNA sequence was submitted to NCBI under accession number PP874720. This sequence was later blasted on NCBI and used for molecular and phylogenetic analysis.

Isolation of bacterial strain

To isolate the entomopathogenic bacteria (EPB) associated with the IJs of the isolated EPN species, larvae of *Galleria mellonella* Linneaus (Lepidoptera: Pyralidae) were infested with 100 IJsLarva^{-1}. Three to four days later, the insect cadavers were surface-sterilized and dissected under aseptic conditions in a laminar flow hood using a sterilized surgical blade. The bacteria-digested internal organs were spread onto Lysogeny Broth (LB) agar plates and incubated at 28°C for 24-48 hours. *Xenorhabdus*–like colonies were then streaked on fresh LB agar plates until monocultures were obtained (Bhat *et al*., 2023; Machado *et al*., 2023). A single primary-form colony was selected and used for further experiments. Bacteria primary forms were determined by examining colony morphology, texture, pigment and production. The strains were further sub-cultured and maintained on LB agar plates at 28°C.

Phenotypic and biochemical characterization of bacteria

The phenotypic characteristics of the isolated bacteria were observed by growing them on sterile plates of nutrient bromothymol blue-triphenyltetrazolium chloride agar (NBTA) (containing of 25 mg/L of bromothymol blue, 4 mg/L of 2,3,5-triphenyltetrazolium chloride, and 20 g/L of nutrient agar) (Akhurst, 1980) and MacConkey agar

plates. The adsorption properties towards neutral red and bromothymol blue (BTB) were particularly noted. Gram staining was performed using 1 minute of Crystal Violet, 1 minute of Iodine mordant, 30 seconds of 95% ethanol, and 1 minute of Safranin O. Optimal bacterial growth temperatures were assessed at 20°C, 24°C, 28°C, 30°C, 37°C, and 42°C. Bacterial growth in saline conditions was evaluated across three NaCl concentrations: 1% (standard LB medium), 2%, and 3%. Growth across a range of pH levels was tested at pH 3, 5, 7 (standard LB medium), 8, and 9. These assessments were conducted in 15 mL conical centrifuge tubes inoculated with 5 mL of LB broth and 0.1 mL of an overnight bacterial culture, followed by incubation at 28°C and 180 rpm for 24 hours (Machado *et al.*, 2022). Antibiotic resistance was assessed on LB agar enriched with tetracycline, vancomycin, or gentamicin at a concentration of 30 mg/L. The overnight bacterial culture was standardized to a cell density of 0.5 McFarland using a DEN-1B McFarland densitometer (Biosan, Riga, Latvia) and 0.85% NaCl. The calibrated bacterial suspension (100 µL) was plated on the supplemented LB agar and incubated at 28°C for 24 hours. All experiments were conducted in triplicate.

Biochemical characterizations were conducted in duplicate using the Hi-Media kit, KB003 Hi25 Enterobacteriaceae Identification Kit (Mumbai, India), specifically designed for characterizing Gram-negative Enterobacteriaceae species. The kit was stored at 4°C before use. During the incubation period, metabolic changes in organisms caused a spontaneous color shift in the medium or through the addition of a reagent. An independent oxidase and catalase test was conducted using the oxidase reagent disc and H2O2, respectively. In the current investigation, the Enterobacteriaceae Identification Kit comprised 24 biochemical tests: 11 carbohydrate utilization tests, and 13 standard biochemical tests. For biochemical testing, EPB from phase I cultures on NBTA media were transferred to 5000 µL of heart infusion broth (Hi-media). The cultures were incubated overnight at 28°C, and 0.05 mL aliquots from each culture were added to each of the kit's 24 wells. After incubating the cultures for 24 hours at 28°C, 50 µL aliquots were added to each of the 24 wells in the Enterobacteriaceae Identification kit. The oxidase and catalase tests were conducted with 16 hour-old liquid LB-bacterial culture (Bhat *et al.*, 2019). The oxidase test involved rubbing a bacterial colony on the oxidase disc provided with the kit. The catalase test was executed using the slide method with 3% (v/v) hydrogen peroxide (H2O2). The kits were processed according to the manufacturer's instructions, and variations in the medium's color were recorded as either positive (+) or negative (-). Chemicals and reagents included in the kits were applied as directed.

Molecular characterization

The bacteria were molecularly characterized using the 16S rRNA gene. For this purpose, genomic DNA was extracted from a single colony of bacteria using the GenElute Bacterial Genomic DNA Kit (Sigma–Aldrich, Switzerland), following the manufacturer's instructions. The 16S rRNA amplification was performed using universal primers 10 F: 5′ -AGTTTGATCATGGCTCAGATTG-3′ (forward) and 1507R: 5′ -TACCTTGTTACGACTTCACCCCAG-3′ (reverse) (Sandström *et al.*, 2001). The PCR product was composed of 1 µL of each forward and reverse primer, 3 µL of DNA extract, 12.5 µL Dream Taq Green PCR master mix 2X (Thermo Scientific), and 7.5 µL nuclease free ddH2O. The PCR profiles included: 1 cycle for 60 seconds at 94°C; followed by 36 cycles for 60 seconds at 94°C, for 60 seconds 55°C and for 120 seconds at 72°C; with a final extension for 10 minutes at 72°C. The amplified products were examined using 1% agarose gel electrophoresis and subsequently sent to Bioserve Pvt. Ltd. in Hyderabad for sanger sequencing. The sequencing results were BLASTed on NCBI, and the assembled sequence was submitted to GenBank under accession number PP775800.

Sequence alignment and phylogenetic analyses

The sequences were edited and compared to those already in GenBank using the National Centre for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLASTN) (Altschul *et al.*, 1990). An alignment of the respective sequences of the nematodes and associated steinernematid species (*carpocapsae* group) was made for the ITS rRNA regions using default MUSCLE settings (Edgar, 2004) in Mega 11, (Tamura *et al.*, 2021), and manually enhanced in BioEdit (Hall, 1999). The pairwise distances were computed using Mega 11 (Tamura *et al.*, 2021). Every character was assigned equal weight, and any gaps were considered missing data. *Steinernema abbasi* was used as the out-group taxa to root the trees. The phylogenetic tree was created using the Maximum Likelihood Tree method (Felsenstein, 1981) in Mega 11 with the General Time Reversible model (GTR+G) algorithm. The tree was generated in the Mega 11, and the Newick format tree containing branch

lengths and bootstrap values was saved. Using the Newick file, the tree was then edited in the Interactive Tree of Life (iTOLv6) (Letunic & Bork, 2021).

The bacteria sample and sequences from similar species of bacteria were aligned for 16S rRNA genes using default MUSCLE settings in Mega 11, (Edgar, 2004; Tamura *et al*., 2021) and manually enhanced in BioEdit (Hall, 1999). The phylogenetic tree was obtained by the Maximum Likelihood Tree method (Felsenstein, 1981) in Mega 11 (Tamura *et al*., 2021) and *Photorhabdus stackebrandtii* (An & Grewal, 2010) Machado et al. (2018) was used as out-group taxa to root the trees.

Results and discussions

Characterization of nematode

Blastn anlaysis of the ITS rRNA sequence of the present nematode isolate showed 100% similarity with already described *Steinernema siamkayai* from different geographical regions. Pairwise alignment of the ITS rRNA sequence of the present isolate showed no nucleotide differences with type population (Stock, 1998), confirming it as the same species. Phylogentic analysis based on ITS rRNA gene sequences supported this molecular data. The ITS rRNA based phylogenetic tree showed that *Steinernema* sp. LD_CU is conspecific with *S. siamkayai*, forming a monophyletic clade. Together, they formed a sister clade with *S. huense* (Phan *et al*., 2014), *S. cumgarense* (Phan *et al*., 2006), *S. tami* (Luc *et al*., 2000), and *S. minutum* (Maneesakorn *et al*., 2010) (Fig. 1). The isolate *Steinernema* sp. LD_CU had 100% sequence similarity and zero total character difference in the ITS rRNA gene, indicating a high degree of similarity with the *S. siamkayai*. With the other species of "*carpocapsae* group", it showed sequence similarity scores ranging from 87% to 98%, and differs in 16-90 nucleotide positions (Table 1). The phylogenetic tree also indicated other sequences with lower scores when the sequence of validly published *Steinernema* species were compared with that of *S. siamkayai* LD_CU (Fig. 1).

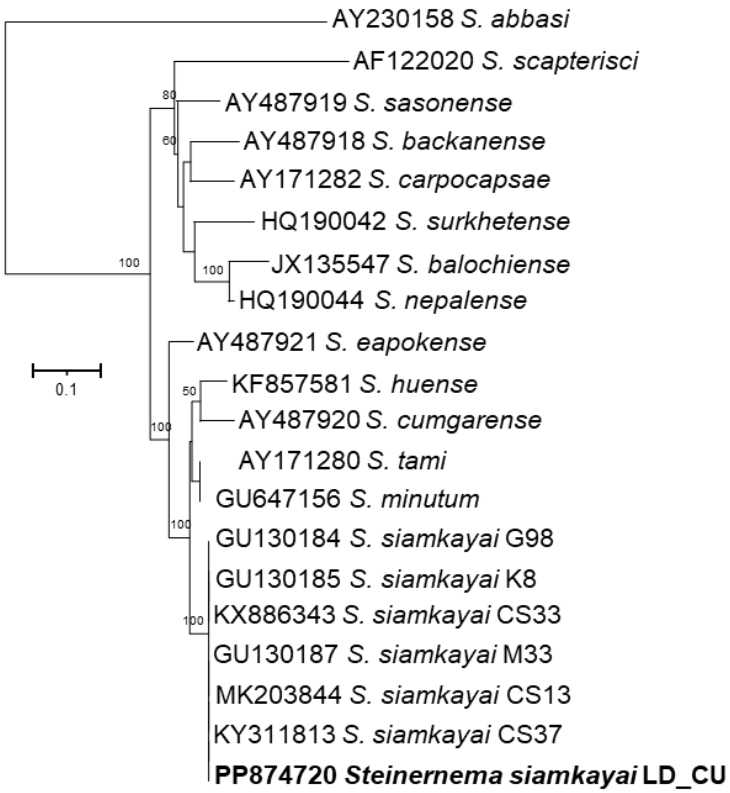

Fig. 1. Maximum-likelihood phylogenetic tree of *Steinernema* EPNs strains reconstructed from ITS rRNA gene sequences. The bootstrap value is represented by numbers at nodes and the bar represent average nucleotide substitutions per sequence position.

Table 1. Pairwise distances of the ITS rRNA gene sequences between *Steinernema siamkayai* and other species of the "*carpocapsae*" group.

S. No.	ITS rRNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	**PP874720 *S. siamkayai***		**0**	**16**	**25**	**27**	**28**	**30**	**49**	**51**	**53**	**54**	**57**	**58**	**90**
2	KY311813 *S. siamkayai*	**100**		15	24	26	29	29	51	51	53	56	57	58	90
3	GU647156 *S. minutum*	**98**	98		22	24	16	26	51	46	54	58	56	53	89
4	AY487920 *S. cumgarense*	**96**	96	97		28	32	35	58	60	61	65	63	64	97
5	KF857581 *S. huense*	**96**	96	96	96		30	34	60	57	58	67	60	67	92
6	AY171280 *S. tami*	**96**	96	98	95	95		36	58	55	61	65	63	67	95
7	AY487921 *S. eapokense*	**95**	96	96	95	95	94		43	46	43	47	41	53	90
8	HQ190044 *S. nepalense*	**92**	92	92	91	90	91	93		36	41	13	39	38	82
9	AY487919 *S. sasonense*	**92**	92	93	90	91	91	93	95		34	41	39	47	81
10	AY171282 *S. carpocapsae*	**92**	92	91	90	91	90	93	94	95		46	39	52	80
11	JX135547 *S. balochiense*	**91**	91	91	89	89	89	93	98	94	93		42	47	84
12	AY487918 *S. backanense*	**91**	91	91	90	90	90	94	94	94	94	94		43	84
13	HQ190042 *S. surkhetense*	**91**	91	92	90	89	89	92	94	93	92	93	93		89
14	AF122020 *S. scapterisci*	**84**	84	84	82	84	83	84	86	86	86	85	85	84	

Below diagonal: percentage similarity; above diagonal: total character differences.

Phenotypic and biochemical characterization of bacteria

The bacterial taxa examined in this study consist of Gram-negative bacilli that demonstrate dye absorption on NBTA plates. EPB exhibit two principal stages, designated as Phase I and Phase II, each characterized by distinct interactions with BTB and neutral red dyes. Phase I bacteria, characterized by their BTB adsorption, typically form colonies with a blue-green color on agar plates, while Phase II bacteria produce colonies with a reddish coloration due to the conversion of tetrazolium chloride into formazan. This chromatic differentiation, whether red or blue-green, facilitates the identification of Phase I and Phase II bacterial colonies across various growth phases (Fukruksa et al., 2017; Machado et al., 2024).

For the assessment of neutral red adsorption, bacterial cultures were grown on MacConkey agar for 24 to 48 hours. Phase I EPB displayed reddish-brown colonies with significant neutral red adsorption, whereas Phase II EPB formed colonies with a pale yellow or off-white appearance (Machado et al., 2023). EPB exhibited growth across a temperature spectrum of 20°C, 24°C, 28°C, 30°C, and 37°C, but not at 42°C. Additionally, EPB were able to proliferate in media containing 1%, 2%, and 3% NaCl, and were viable at pH levels of 3, 5, 7, 8, and 9. Antibiotic susceptibility testing revealed that the present bacterial strain was susceptible to tetracycline and gentamicin, but resistant to vancomycin.

The biochemical tests indicated that the bacteria exhibit characteristics similar to *Xenorhabdus stockiae*. The colony was negative for oxidase, catalase, nitrate reductase, and O-Nitrophenyl-β-D galactopyranoside (ONPG). They were positive for urea and esculin hydrolysis, and show motility. On nutrient agar, they showed brownish pigmentation, neutral red adsorption on MacConkey agar forming red colonies, and a negative reaction for the Voges-Proskauer assay. The isolated strain was negative for the methyl red, tryptophan deaminase and indole production assays, and did not utilize ornithine, lysine, citrate, and malonate. It produced acid from glucose fermentation, but not from lactose, ribose, trehalose, raffinose, saccharose, melibiose, cellobiose, rhamnose, adonitol, xylose, and arabinose. Acid production from myo-inositol was negative. In addition, the bacteria did not produce hydrogen sulphide (H2S) or acetoin, and tested negative for phenylalanine deaminase (Table 2). Overall, these biochemical characteristics provide a comprehensive profile of the bacterial isolates, aligning them closely with the known properties of *Xenorhabdus stockiae* (Akhurst, 1983; Machado et al., 2024; Tailliez et al., 2006). This detailed profiling is crucial for accurate identification and understanding of the bacterial species.

Molecular characterization of bacteria and its phylogenetic analysis

The isolated bacteria were molecularly characterized using 16S rRNA gene sequences. BLAST nucleotide analysis revealed that the isolated *Xenorhabdus* bacterial strain exhibited 100% similarity to the previously described *X. stockiae* (Tailliez et al., 2006), showing complete resemblance, but showed 90% similarity with *X. innexi* (Lengyel et al., 2005), highlighting a significant divergence.The phylogenetic tree also displayed other sequences with lower scores when the sequence of each officially published *Xenorhabdus* species was compared with that of the isolated *Xenorhabdus* bacterial strain (Fig. 2). These findings suggest that the isolated *Xenorhabdus* bacterial strain is closely related to *X. stockiae* VP-2016b and distinctly different from *X. innexi* DSM 16336T and other species within the *Xenorhabdus* genus.

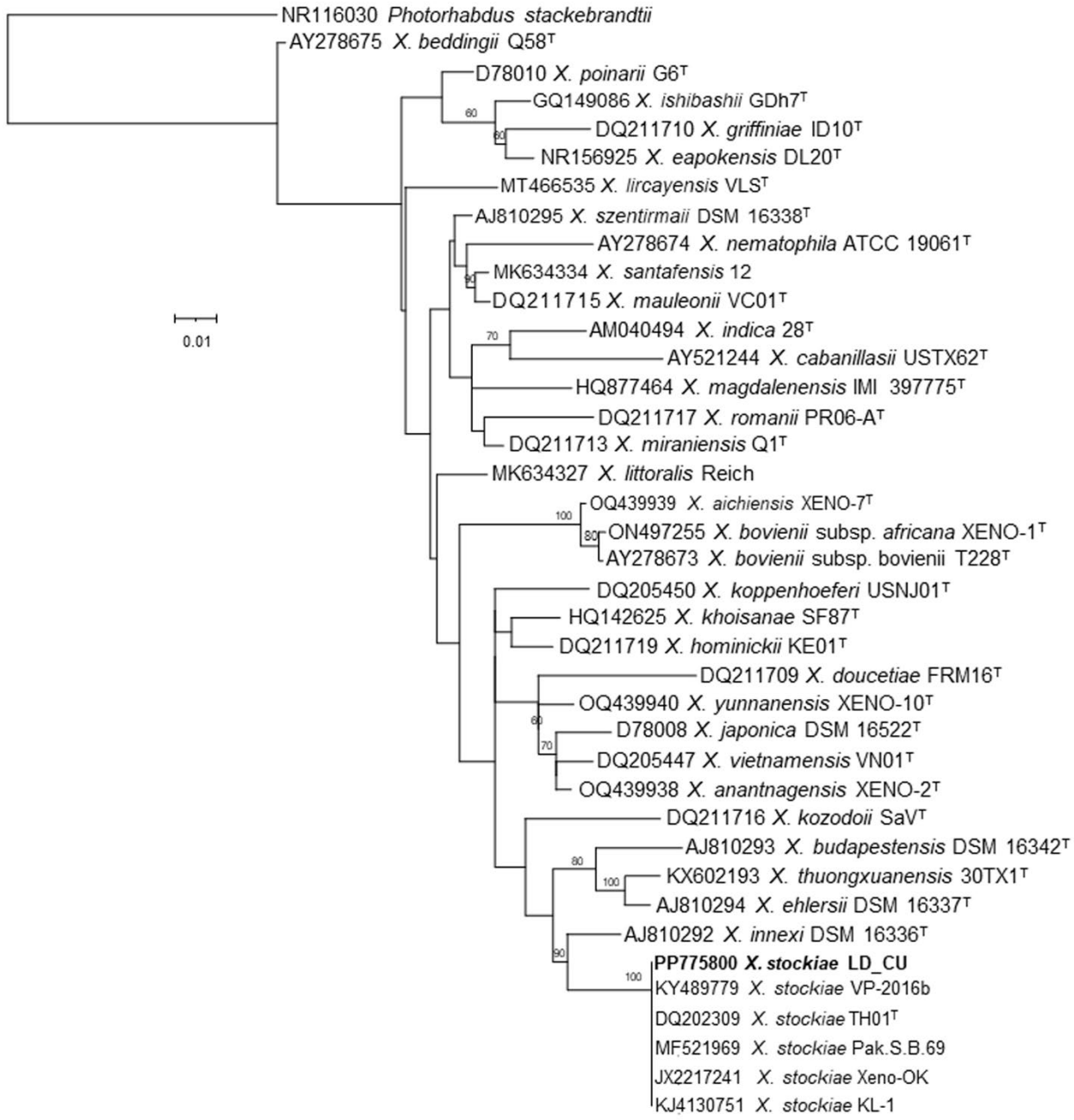

Fig 2. Maximum-likelihood phylogenetic tree of *Xenorhabdus* bacterial strains reconstructed from 16S rRNA gene sequences. The bootstrap value is represented by numbers at nodes and the bar represent average nucleotide substitutions per sequence position.

Compared to with *X. stockiae*, the 16S rRNA region of isolated *Xenorhabdus* bacterial strain shares 100% similarity and shows no nucleotide difference, while showing differences of 27–46 bp, resulting in sequence similarity values of 96–98% with other *Xenorhabdus* species (Table 3).

Conclusion

In the present study, the molecular and phenotypic analyses confirm that the nematode isolate *Steinernema* sp. LD_CU is conspecific with *Steinernema siamkayai*, demonstrating 100% sequence similarity and forming a monophyletic clade with related species in the phylogenetic tree. The isolate exhibits no nucleotide variation from the type population, underscoring its close genetic relationship with *S. siamkayai*.

Table 2. Biochemical characteristics of *Xenorhabdus stockiae* isolated from *Steinernema siamkayai*.

S. No.	Tests	Results	S. No.	Tests	Results
1.	O-Nitrophenyl-β-D galactopyranoside (ONPG)	-	18.	Arabinose	-
2.	Lysine utilization	-	19.	Xylose	-
3.	Ornithine utilization	-	20.	Adonitol	-
4.	Urea hydrolysis	+	21.	Rhamnose	-
5.	Phenylalanine deaminase	-	22.	Cellobiose	-
6.	Nitrate reduction	-	23.	Melibiose	-
7.	H_2S production	-	24.	Saccharose	-
8.	Citrate utilization	-	25.	Raffinose	-
9.	Voges Proskauer	-	26.	Trehalose	-
10.	Methyl red	-	27.	Glucose	+
11.	Indol	-	28.	Lactose	-
12.	Malonate utilization	-	29.	Oxidase	-
13.	Esculin hydrolysis	+	30.	Ribose	-
14.	Myo-inositol	-	31.	Bioluminescence	-
15.	Dye absorption BTB from NBTA	+	32.	Pigmentation	Brownish
16.	Neutral red MaConkey Agar	Red	33.	Motility	+
17.	Tryptophan deaminase	-	34.	Catalase	-

+ = positive reaction, – = negative reaction.

Table 3. Pairwise distances in base pairs of the 16S rRNA gene sequences of *Xenorhabdus* and isolated bacterial strain. Data for present species is in bold.

S. No.	16SrRNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***X. stockiae***		**0**	**27**	**31**	**32**	**33**	**34**	**34**	**35**	**35**	**36**	**39**	**39**	**39**	**39**	**46**
2	*X. stockiae*	**100**		27	31	32	33	34	34	35	35	36	39	39	39	39	46
3	*X. hominickii*	**98**	98		24	16	34	35	34	31	30	36	28	21	38	38	30
4	*X. thuongxuanensis*	**98**	98	98		21	26	38	27	29	12	21	24	25	38	33	38
5	*X. khoisanae*	**98**	98	99	98		36	38	37	25	31	38	23	19	42	30	37
6	*X. eapokensis*	**97**	97	97	98	97		25	44	21	33	17	44	44	31	45	41
7	*X. santafensis*	**97**	97	97	97	97	98		44	18	41	30	33	35	31	45	39
8	*X. innexi*	**97**	97	97	98	97	97	97		41	27	41	35	42	42	44	50
9	*X. miraniensis*	**97**	97	98	98	98	98	99	97		32	24	32	32	26	41	31
10	*X. ehlersii*	**97**	97	98	99	98	97	97	98	98		30	28	34	40	36	43
11	*X. ishibashii*	**97**	97	97	98	97	99	98	97	98	98		48	46	28	41	39
12	*X. anantnagensis*	**97**	97	98	98	98	97	97	97	98	98	96		14	41	34	44
13	*X. yunnanensis*	**97**	97	98	98	99	97	97	97	98	97	96	99		44	40	43
14	*X. indica*	**97**	97	97	97	97	98	98	97	98	97	98	97	97		42	45
15	*X. kozodoii*	**97**	97	97	97	98	96	96	97	97	97	97	97	97	97		47
16	*X. aichiensis*	**96**	96	98	97	97	97	97	96	98	97	97	97	97	96	96	

Below diagonal: percentage similarity; above diagonal: total character differences.

In parallel, the bacterial symbionts associated with this nematode, identified as *Xenorhabdus stockiae*, display consistent biochemical and molecular profiles with previously described strains. The isolate shows a 100% sequence match with *X. stockiae* and notable divergence from *X. innexi*, reflecting its precise taxonomic placement. This comprehensive characterization enhances our understanding of the nematode-bacterium association and underscores the need for detailed profiling in ecological and biotechnological applications.

Author's Contributions

Conceptualization: **Ladoi Drema** and **Aashaq Hussain Bhat**; Methodology: **Ladoi Drema**, **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Formal analysis: **Ladoi Drema**, **Aashaq Hussain Bhat**; Investigation: **Ladoi Drema**, **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Draft preparation: **Ladoi Drema**, **Aashaq Hussain Bhat**; Fina review and editing: **Ladoi Drema**, **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Visualization: **Ladoi Drema**, **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Validation: **Aashaq Hussain Bhat**; Supervision: **Aashaq Hussain Bhat**; Project administration: **Aashaq Hussain Bhat**. All the authors read and approved the manuscript.

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Data Availability Statement

All data supporting the findings of this study are available within the paper.

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Ethics Approval

Insects, nematodes and bacteria were used in this study. All applicable international, national, and institutional guide lines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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رویکردی تلفیقی در تعیین مشخصات *Xenorhabdus stockiae* و *Steinernema siamkayai* با استناد به داده‌های مولکولی و فنوتیپی

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چکیده

در این تحقیق، جدایه LD_cu نماتود بیمارگر حشرات و باکتری همزیستش، تعیین مشخصات شدند. تجزیه و تحلیل BLASTn توالی ITS نماتد، همسانی ۱۰۰٪ این نمونه با *Steinernema siamkayai* را نشان داد. هم ترازی جفت جفت توالی‌ها نشان داد که هیچگونه اختلاف نوکلئوتیدی بین ناحیه ژنی ITS این نماتود با گونه *S. siamkayai* وجود ندارد. ارزیابی شجره شناسی بر اساس توالی ژن ITS نیز این یافته را تایید نمود و جدایه LD_CU و گونه *S. siamkayai* در یک شاخه تک نیا قرار گرفتند و با گونه های *S. cumgarense* ،*huense* ،*S. tami* و *S. minutum* یک شاخه خواهری شکل دادند. آزمون های بیوشیمیایی نشان داد که باکتری همزیست، گونه *Xenorhabdus stockiae* می‌باشد. جدایه باکتری از لحاظ واکنش های اکسیداز، کاتالاز، احیای نیترات، او نیتروفنول-بتا- دی گالاکتوپیرانوسید(ONPG) ، متیل قرمز، تریپتوفان دآمیناز، تولید ایندول، اورنیتین، لیزین، سیترات، مالونات، سولفید هیدروژن، استوئین، و فنیل آلانین دآمیناز، منفی بود. اما واکنش آن برای هیدرولیز اوره و اسکولین، تحرک و تولید اسید از تخمیر گلوکز، مثبت است. جدایه باکتری جذب قرمز خنثی را روی MacConkey آگار نشان دادند و کلنی آن، رنگدانه ای قهوه ای را روی محیط آگار تشکیل دادند. شناسایی مولکولی با استفاده از توالی ژن 16S موید هم‌سانی ۱۰۰ درصدی با *X. stockiae* بود در حالی که با *X. innexi* ۹۰ درصد همسانی نشان داد که این حکایت از واگرایی قابل توجه این گونه باکتری از سایر گونه های *Xenorhabdus* است. تجزیه و تحلیل شجره‌شناسی نشان داد که جدیه باکتری ارتباط نزدیکی با *X. stockiae* VP-2016b دارد ولی از گونه *X. innexi* DSM 16336T تمایز قابل توجهی دارد. هم ترازی دو به دو توالی 16S نشان داد هیچ تفاوت نوکلئوتیدی بین این جدایه باکتری و *X. stockiae* وجود ندارد. این مطالعه، شناسایی دقیق و جامعی از نماتود بیمارگر حشرات، *Steinernema siamkayai* و همزیست باکتریایی آن، *Xenorhabdus stockiae* بوده و اطلاعات ارزشمندی را به موضوع طبقه‌بندی و فیلوژنی این گروه اضافه می کند.

کلمات کلیدی: تعیین مشخصات،BLAST ، ITS rRNA، 16S rRNA، نماتد بیمارگر حشرات، باکتری همزیست، بیماری شناسی حشرات

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